SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Animals

Poldip2 gene trap mice on a C57BL/6 background were produced by the Texas A&M Institute for Genomic Medicine. Since homozygous Poldip2 depletion in gene trap mice is embryonically lethal,[22] heterozygous Poldip2-deficient mice ($Poldip2^{+/-}$) were used in this study, and wild-type mice ($Poldip2^{+/+}$) were used as controls. Poldip2 myeloid specific knockout mice were generated by crossing a LysM-Cre strain (C57BL/6 background) obtained from The Jackson Laboratory with our newly created Poldip2 floxed mice (C57BL/6 background).[23] Mice with Poldip2 deficiency in myeloid cells are hereafter designated $myePoldip2^{-/-}$, while their littermates without Poldip2 deficiency are designated $myePoldip2^{+/+}$. Mice were genotyped for Cre using a real-time PCR assay with melting curve analysis from The Jackson Laboratory and for floxed Poldip2 using a standard three-primer PCR method.[23] Hematopoiesis of these genetic modified mice was evaluated by assessing complete blood count from cardiac puncture blood samples. Mice received LabDiet chow 5053 and water ad libitum. All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee at Emory University.

LPS-induced ARDS model

Adult male and female (age 2.5-3.5 months) $myePoldip2^{+/+}$ and $myePoldip2^{-/-}$ mice were randomly divided into control and LPS groups using the block randomization method. Animals in the LPS group received an intraperitoneal (i.p.) injection of LPS (18 mg/kg) from Escherichia coli 0111: B4 (InvivoGen, tlrl-eblps) diluted in sterile normal saline. An equal volume of sterile normal saline was given to mice in the control group. Mice were monitored for signs of distress or discomfort. Eighteen hours after injection, rectal temperature was measured, and mice were euthanized by CO_2 asphyxiation for either bronchoalveolar lavage (BAL) or lung tissue collection. Mice that died before BAL and lung tissue collection (2 in $myePoldip2^{+/+}$ group and 1 in $myePoldip2^{-/-}$ group) were excluded. No difference between sexes was detected.

Bronchoalveolar lavage (BAL)

Mice were first euthanized by CO_2 inhalation and tracheas were exposed and cannulated using a 20-gauge lavage needle. For assessment of total cell counts in BAL, three lavages with 1 ml each of PBS containing 2 mM EDTA were injected in the tracheal lavage needle and recovered as previously described.[21] The volume recovered was measured for normalization and then centrifuged at 300 g, 4°C for 10 min. Pellets were resuspended in Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (HBSS-, Gibco, #14175) for either total BAL cell counts or flow cytometry. For total cell counting, pellets were processed with RBC lysis buffer (Alfa Aesar, #J62150) prior to counting using a Bio-Rad TC20 Automated cell counter.

Flow cytometry

For neutrophil identification in BAL, samples were processed with RBC lysis buffer right after collection and spun down at 300 g for 5 min at room temperature. The cell pellet was resuspended in HBSS containing 2 mM EDTA (RPI, #E14000) and cells were blocked with anti-mouse CD16/32 (ThermoFisher, #14-0161-82) for 15 min on ice, followed by incubation with anti-mouse antibodies on ice for 30 min in the dark. Antibodies were APC-Ly6G (ThermoFisher, #17-9668-82), eFluor450-CD11b (ThermoFisher, #48-0112-82), APC-eFluor780-CD45 (ThermoFisher, #47-0451-82). For evaluation of integrin surface expression, isolated neutrophils were labeled with eFluor450-CD11b or FITC-CD18 (Biolegend, #101405). For evaluation of L-selectin shedding, neutrophils were stained with CD62L (ThermoFisher, #11-0621-82). Flow cytometry was performed on a Cytek Aurora flow cytometer (Cytek Biosciences). Data were analyzed with Flowjo software (Tree star), and leukocytes were gated as $CD45^+$; myeloid cells were further gated as $CD11b^+$ and neutrophils were identified as $Ly6G^+$. For complete blood cell counts, white blood cell differential counting was achieved using a Hemavet 1500 blood analyzer (CDC Technologies, Oxford, CT).

Immunofluorescence, histology and microscopy

Eighteen hours after PBS or LPS treatment, mice were sacrificed using CO_2 asphyxiation, and lungs were injected with 10% formalin through the trachea using a 21-gauge lavage needle. Lungs were then dissected, placed in 10% formalin for 24 h, and transferred to 70% ethanol, paraffin embedded and sectioned (5 µm). Fixed paraffin lung sections were used for hematoxylin & eosin (H&E) staining and immunostaining. For H&E staining, paraffin sections were deparaffinized then rehydrated in a series of xylene, ethanol and PBS. Hematoxylin and eosin staining were then performed according to standard protocols[24]. Pictures were taken under a NanoZoomer-SQ Digital slide scanner (Hamamatsu) at 20X magnification. For immunostaining, 10 µg/ml proteinase K (Abcam, #ab64220) was applied for antigen retrieval (30 min, room temperature), and sections were blocked with 3% BSA containing 3% normal goat serum (Vector, #S-1000) prior to incubation with primary antibody against Ly6G (1:200 diluted, Abcam, #ab2557) overnight at 4°C. Sections were then stained with Alexa Fluor 568 goat anti-rat IgG (1:500 diluted, Invitrogen, #A11077), and mounted with vectashield mounting medium with DAPI (Vector, H-1200). Pictures were taken on a Zeiss LSM 800 Airyscan microscope at 20X magnification, and the ratio of Ly6G and DAPI positive area was calculated using ImageJ for assessment of neutrophil infiltration. Data were quantified from three fields of one section per animal, and 5 animals per group.

Bone marrow neutrophil isolation and purification

Primary bone marrow neutrophils were isolated as described[25] and purified by the immunomagnetic negative selection technique, using an Easysep Mouse Neutrophil Enrichment Kit (Stemcell Technologies, #19762). Briefly, both ends of the femur and tibia were dissociated after euthanasia and bone marrow cells were flushed using a 27-gauge needle and 10 ml syringe filled with HBSS- supplemented with 2% FBS (Benchmark, #100-106) and 2 mM EDTA. The cell suspension was passed through a 70 µm mesh nylon strainer to remove clumps of cells and debris and centrifuged at 300 g for 10 min. Resuspended cells were then processed following the manufacturer's instructions to get purified neutrophils. Neutrophil purity was always higher than 85%

and was assessed by staining with the fluorophore-labeled neutrophil marker, Ly6G, followed by flow cytometry analysis.

Cell culture

Primary rat pulmonary microvascular endothelial cells (RPMECs; Cell Biologics, #RN-6011) and primary mouse lung microvascular endothelial cells (MLMECs; Cell Biologics, #C57-6011) were cultured on plates pre-coated with 0.1% gelatin from bovine skin (Sigma; Cat No. G6650). Endothelial cell medium was supplemented with 2% fetal bovine serum, endothelial cell growth factors, and antibiotics (Cell Biologics; #M1266 for RPMECs, #M1168 for MLMECs). Cells were used from passage 4 to 6.

Static adhesion assay

Isolated bone marrow neutrophils were spun down at 300 g, 20°C for 5 min, resuspended in serum-free Dulbecco's Modified Eagle's Medium (DMEM; Sigma, #D5671) containing 0.2% BSA (Sigma, #3117332001), 2 mM L-glutamine (ThermoFisher, #25030), and Hoechst 33342 (1:2000, ThermoFisher, #62249), and incubated at 37°C, 5% CO_2 for 10 min. Cells were then washed twice with DMEM, and resuspended at $1x10^6$ cells/ml. MLMECs were seeded onto a 24-well plate and treated with 10 ng/mL TNF-α once a monolayer was formed. After 6 hours, MLMECs were washed twice with DMEM followed by addition of bone marrow neutrophils (2×10^5 cell per well). Neutrophils and MLMECs were co-incubated for 30 min at 37°C followed by two washes with warm HBSS with Ca^{2+} and Mg^{2+} (HBSS+, Gibco, #14025) and then fixed using 3.7% paraformaldehyde (PFA, Electron Microscopy Science, #15714-S) for 10 min at room temperature. Fixed cells were washed twice with HBSS+ and immediately imaged with an Olympus IX71 inverted fluorescent microscope using the DAPI fluorescence channel at 10x magnification. Three representative pictures were taken per well, and triplicate wells were used for each condition. Images were analyzed with ImageJ software (NIH); stained neutrophil nuclei were counted using the "analyze particles" module to assess firm adhesion to the MLMEC monolayer.

µ-slide chemotaxis

µ-Slide Chemotaxis (ibidi GmbH, #80326) was used for the chemotaxis assay. Each slide contains three chambers, and each chamber consists of one channel for the cells and two reservoirs for the chemoattractant or chemoattractant-free media on either side of the channel. Prior to use, cell medium and µ-Slide were stored in the incubator at 37°C, 5% CO_2 overnight for equilibration. Poldip2$^{+/+}$ and Poldip2$^{+/-}$ bone marrow neutrophils were isolated as described above and incubated in HBSS+ containing Hoechst 33342 (1:2000) at 37°C for 10min, washed twice and then resuspended in HBSS+ at $3x10^6$ cells/ml before seeding into the central channel. Assays were conducted following the manufacturer's instructions. Briefly, the cell suspension (6 µl) was seeded into the channel and the µ-slide was incubated inside a sterile Petri dish for 15 min with a wet tissue to minimize evaporation. Next, both reservoirs were filled with 65 µL chemoattractant-free medium and then fMLP (final concentration 50 µM) was added to one reservoir. Negative and positive control experiments were performed in the other two chambers of the same µ-slide, in which both reservoirs were filled with either chemoattractant-free medium or the same chemoattractant solution as in the chemotaxis experiments. Cell movement was observed using a Leica TCS SP5 II laser

scanning confocal microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany). Time lapse videos were acquired using LAS AF software with a Plan-Neo 10× 0.3NA air objective. After adding 50 μM fMLP, images were captured every minute for a total duration of 2 hours in brightfield mode and DAPI mode (using a 405 nm laser Diode 50 mW with a DAPI filter set). Cell trajectories were analyzed using a customized Python cell tracker software. Distance threshold was set as 30 μm based on the cell trajectories of negative control experiments. Statistical significance ($P<0.05$) was calculated from 3 independent experiments (n=3) where over 60 cells were evaluated in each condition per experiment.

Transmigration assay

For the transmigration assay, 3.0 μm pore size transwell membrane inserts were utilized. MLMECs (3×10^5) were seeded onto the membrane and after forming a monolayer at 24 hours, they were treated with 10 ng/ml TNF-α or media alone for 6 hours. Hoechst pre-stained neutrophils (0.5×10^6) were added to the upper chamber of each insert and 100nM fMLP or media alone was added to the lower chamber. After 2 hours of incubation at 37℃, non-migrated cells were removed from the upper surface using a wet cotton swab and migrated cells on the lower surface were fixed in 4% PFA for 3 min. Inserts were then washed 3 times with PBS and membranes were cut out from inserts and mounted on slides. Pictures were taken using an Olympus DP71 Digital Microscope at 10X and migrated neutrophils were counted by a blinded observer in six fields for each well using ImageJ software (NIH). Neutrophils transmigrated into the media were also collected and counted manually using hemocytometer.

Soluble ICAM-1 binding assay

The soluble ICAM-1 binding assay was used to assess beta integrin activation in neutrophils[26]. Bone marrow cells were isolated from myePoldip2$^{+/+}$ and myePoldip2$^{-/-}$ mice and suspended in HBSS+. Cells were exposed to 2 mM EDTA (as negative control), 5 mM $MnCl_2$ (Sigma, #M1787) or an equal volume of HBSS+, in the presence of 10 μg/ml recombinant mouse ICAM-1-Fc chimera (R&D Systems, # 796-IC-050) and 10 μg/ml PE-conjugated anti-human IgG1 Fc (ThermoFisher, #12-4998-82) for 10 min at 37℃. Cells were fixed on ice with 3.7% PFA for 30 min and then labeled with APC-conjugated anti-Ly6G (ThermoFisher, #17-9668-82) to identify neutrophils. ICAM-1 binding was measured using flow cytometry measurement of PE mean fluorescence intensity in the neutrophil subset.

SYTOX Green assay

The SYTOX green assay was used to quantify the abundance of extracellular DNA as a surrogate of neutrophil extracellular trap (NET) formation as previously described.[27] Poldip2$^{+/+}$ and Poldip2$^{+/-}$ neutrophils were isolated as described above. Fifty thousand cells per well were plated in a 96 well black clear-bottom plate and incubated at 37°C for 1 h. Cells were then stimulated with 324 nmol of phorbol 12-myristate 13-acetate (PMA; Sigma, #P8139) or 50 μg/ml of LPS (Sigma, #L4391). SYTOX green dye (5 μM, Invitrogen, #S7020) was added to each well and the fluorescence was read with filter setting at 485-nm excitation/525-nm emission using a Synergy H1 Microplate Reader and Gene5 software (Biotek, Winooski, VT). Fluorescence was read every 15 min for a total of 90 min at 37°C. Four replicates were measured in each experiment. Three

independent experiments were performed. Both the time course curves, as well as final fluorescence at 90 min were analyzed.

Reactive oxygen species (ROS) production

Bone marrow neutrophils isolated from Poldip2$^{+/+}$ and Poldip2$^{+/-}$ mice were resuspended in HBSS+ and transferred to a 96 well plate (1x10^6 neutrophils per well). A cytochrome C assay was applied for ROS production measurement.[28] Briefly, cytochrome C (100 nM) was added to all wells and 25 units of superoxide dismutase (SOD) was added to control wells. Neutrophils were stimulated with 100 nM PMA. Absorbance was read at 550 nm (absorbance of reduced Cytochrome C) and 490 nm (to control for non-specific absorbance) every minute for 2 hours immediately after application of PMA. The concentration of superoxide was calculated using the following formula [$O_2^{\cdot -}$ (nmol)=[(Abs(X)$_{550}$-Abs(X)$_{490}$) – (Abs(SOD)$_{550}$- Abs(SOD)$_{490}$) /(2.1x10^4 M^{-1}cm^{-1} x 0.294cm)]x10^{9}x10^{-4} L, where X is the well of interest, SOD the corresponding well containing superoxide dismutase, 2.1x10^4 M^{-1}cm^{-1} is the Cytochrome C extinction coefficient, 0.294 is the distance travelled by light (cm), 10^{-4} L is the reaction volume and 10^9 is the conversion coefficient from mol to nmol. The average of 3 independent experiments (2-6 independent wells each) was calculated.

RNA extraction and RT-qPCR

Total RNA was purified with Qiazol (Qiagen, #79306) and the RNeasy Plus kit (Qiagen, #74104). Reverse transcription was performed using Protoscript reverse transcriptase (New England Biolabs) with random primers. The resulting cDNA was amplified with previously validated primers against ribosomal protein LI3A (RPL), Hypoxanthine Phosphoribosyl transferase (HPRT), Interleukin 1 Beta (IL-1β), Interleukin 6 (IL-6), Tumor Necrosis Factor Alpha (TNF-α), C-C Motif Chemokine Ligand 2 (CCL-2/MCP-1), C-X-C Motif Chemokine Ligand 1 (CXCL-1), C-X-C Motif Chemokine Ligand 2 (CXCL-2), and Poldip2 (for primer details please refer to Table 1). RPL and HPRT were used as housekeeping genes as their expression was not affected by LPS treatment. Note that Poldip2 primers can detect messages transcribed from both floxed and Cre-excised alleles. Amplification was performed in 96-well plates using Forget-Me-Not EvaGreen qPCR Master Mix with low ROX (Biotium, #31045) in a QuantStudio 7 instrument (Invitrogen). Data analysis was performed using the mak3i module of the qpcR software library (version 1.4-0)[29,30] in the R-environment.[31] Final result quantification was expressed in arbitrary units.

Enzyme-linked immunosorbent assay (ELISA)

Bone marrow neutrophils isolated and purified from myePoldip2$^{+/+}$ and myePoldip2$^{-/-}$ mice were plated on 24-well plate (3 x 10^6 /600µL/well) and stimulated with LPS (1µg/ml) or media only for 8 hours at 37°C, 5% CO_2. Supernatant was then collected for TNF-α, IL-1β, and IL-6 production quantification. Commercial ELISA kits (R&D, #MLB00C, #M6000B, #MTA00B) were utilized according to the manufacturer's instructions. Five independent experiments were performed, and two replicates were measured in each experiment.

Western blotting

Whole cell lysate was prepared from isolated neutrophils using a lysis buffer described in our previous study:[20] 0.3 M NaCl, 0.2% SDS, 0.1 M Tris base, 1% Triton X-100, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 1 mM PMSF, and Halt phosphatase inhibitor cocktail (ThermoFisher, #78428). Homogenates were centrifuged at 12,000 g at 4°C for 10 min and brought to equal concentration in Laemmli buffer before boiling at 100°C for 10 min. For Pyk2 phosphorylation detection, bone marrow neutrophils were plated onto ICAM-1 coated 6-well plates and stimulated with TNF-α or media alone for the specified time. Neutrophil lysates were then prepared by adding 2x laemlli buffer and boiling for another 5 min. Samples were stored at -20°C until gel loading. Proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes (GE, #10600003) and assessed by blotting with primary antibodies against Poldip2 (Abcam, #ab181841), Pyk2 (CST, #3292S), phosphorylated Pyk2 (CST, #3291S), β-actin (CST, #4970) and vinculin (Sigma, #V4505). Blots were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies [anti-mouse (GE, NA931V) and anti-Rabbit (Cell signaling technology, #70745)]. Detection was performed using enhanced chemiluminescence (ThermoFisher, #32106) and autoradiography film (Genesee scientific, # 30-810L). Detected bands were scanned using an Epson Perfection V800 Photo scanner, and densitometry was performed using ImageJ.

Statistical Analysis

Analyses were carried out using results from 3-10 independent experiments with GraphPad Prism software version 8. Data were represented as medians with their 95% confidence intervals (CI) and analyzed using non-parametric methods, either Mann Whitney or Kruskal-Wallis with Dunnett multiple comparisons. A threshold of $P<0.05$ was considered significant. Micrographs showing average numbers of adherent cells were selected as representative.

Table S1. Major Resources Table - Wild Type and Genetically Modified Animals (in vivo studies).

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	The Jackson Laboratory	C57BL/6J	Both	https://www.jax.org/strain/000664
Mouse	The Jackson Laboratory	C57BL/6	Both	https://www.jax.org/strain/004781
Mouse	Griendling, K.K.	C57BL/6	Both	https://doi.org/10.1371/journal.pone.0247261
Mouse	TIGM	C57BL/6	Both	https://tigmtrack.tamu.edu/tigm-web/pages/publicSearchResult.xhtml?type=%27Gene%27&name=%27Poldip2%27

Antibodies

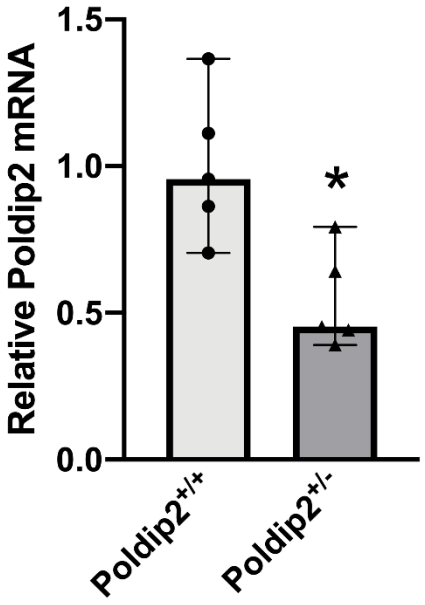
Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Ly6G (Flow cytometry)	ThermoFisher	17-9668-82	1:100		
CD11b (Flow cytometry)	ThermoFisher	48-0112-82	1:100		
CD45 (Flow cytometry)	ThermoFisher	47-0451-82	1:100		
CD18 (Flow cytometry)	Biolegend	101405	1:100		
CD62L (Flow cytometry)	ThermoFisher	11-0621-82	1:100		
Ly6G (Immunofluorescence)	Abcam	ab2557	1:200		
Biotinylated Rat IgG	Vector	BA-9400	1:400		
Human IgG1 Fc (Flow cytometry)	ThermoFisher	12-4998-82	10 µg/ml		
Poldip2	Abcam	Ab181841	1:2000		
β2-integrin	R&D system	AF1730	1:2000		
Vinculin	Sigma	V4505	1:2000		
Phospho-Pyk2 (Tyr402)	Cell signaling technology	3291S	1:1000		
Pyk2	Cell signaling technology	3292S	1:2000		
β-actin	Cell signaling technology	4970	1:5000		

Mouse IgG	GE	NA931V	1:2000		
Rabbit IgG	Cell signaling technology	70745	1:5000		

Cultured Cells

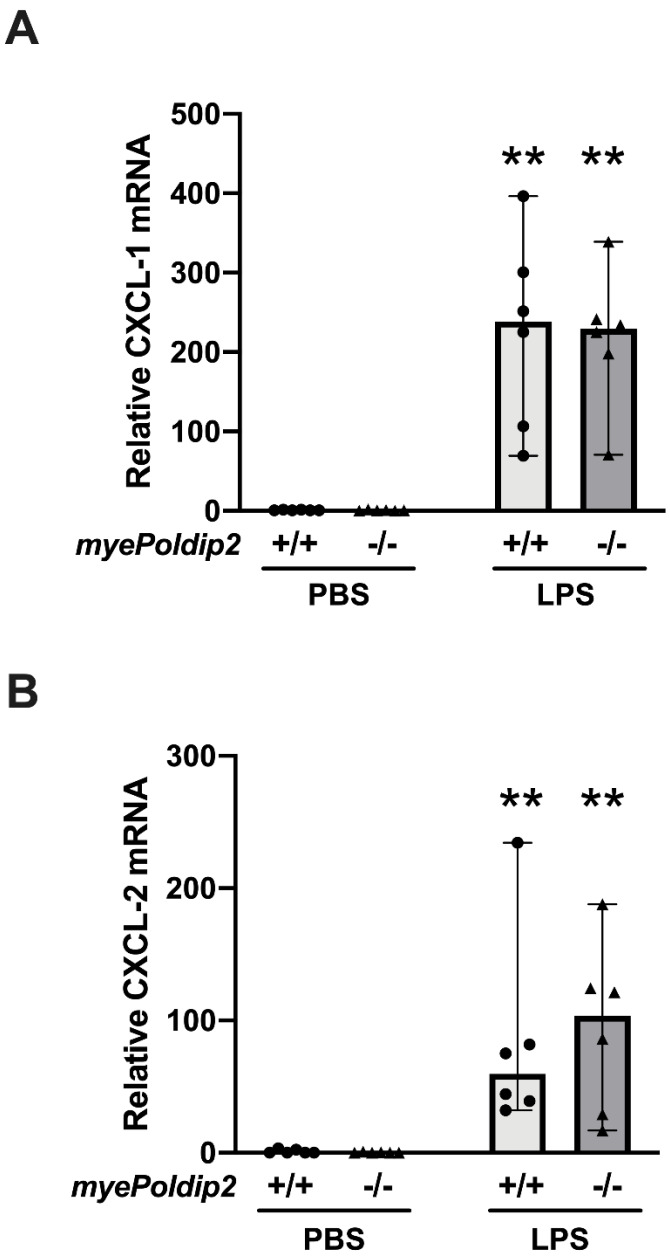
Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Mouse primary lung microvascular endothelial cell	Cell biologics	unknown	C57-6011
Rat primary lung microvascular endothelial cell	Cell biologics	unknown	RN-6011

Figure S1. Poldip2 expression in Poldip2$^{+/-}$ mice.



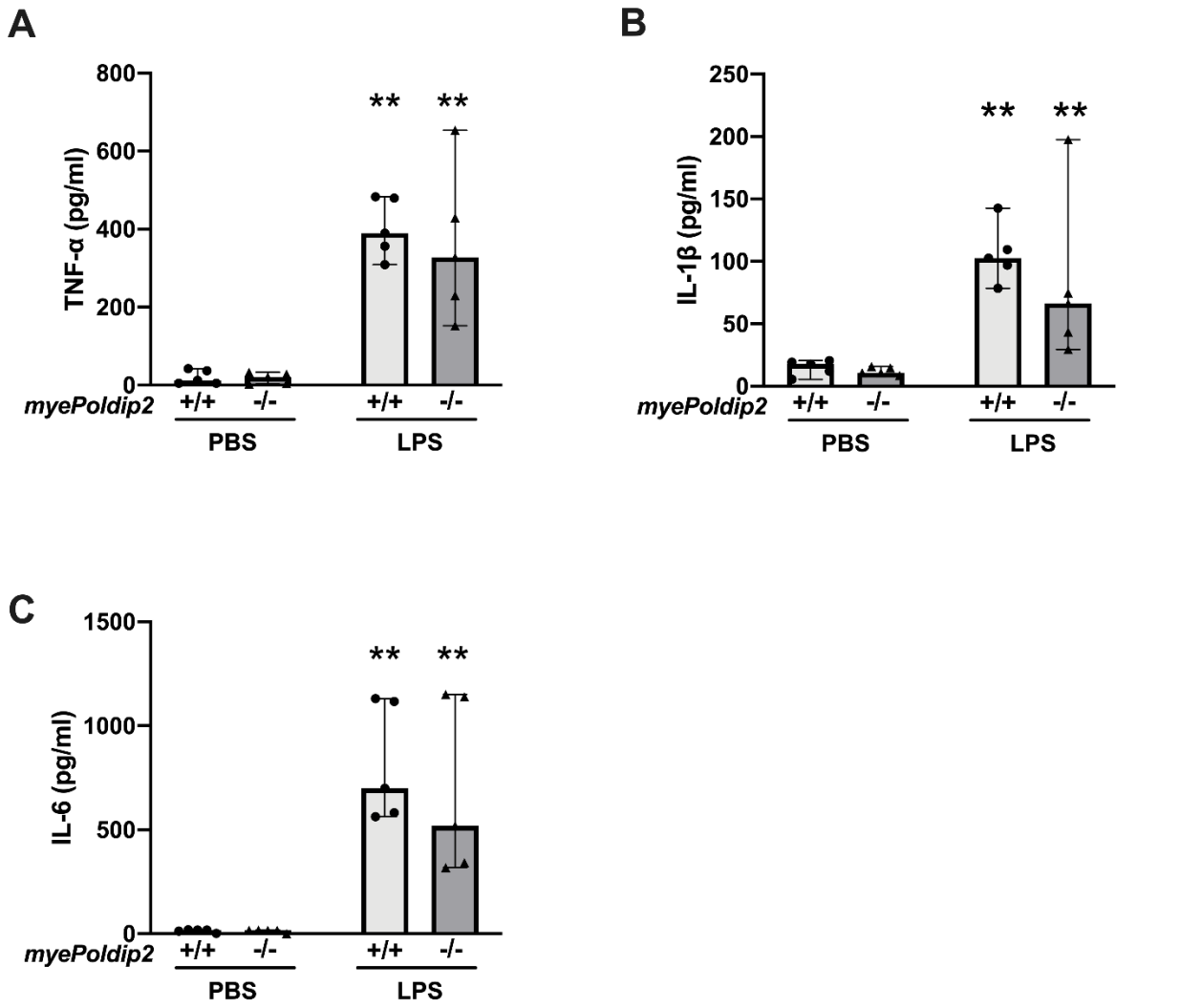
Poldip2 mRNA expression (normalized to RPL and HPRT) in purified neutrophils measured by quantitative RT-PCR. Data represent medians with 95% confidence intervals (n=5); * P<0.05 (Mann Whitney test). Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; RPL, ribosomal protein L13A; HPRT, hypoxanthine guanine phosphoribosyl transferase; and RT-PCR, real time polymerase chain reaction.

Figure S2. Poldip2 knockdown in myeloid cells does not affect LPS-induced upregulation of neutrophil-targeting chemokines in mouse lung.



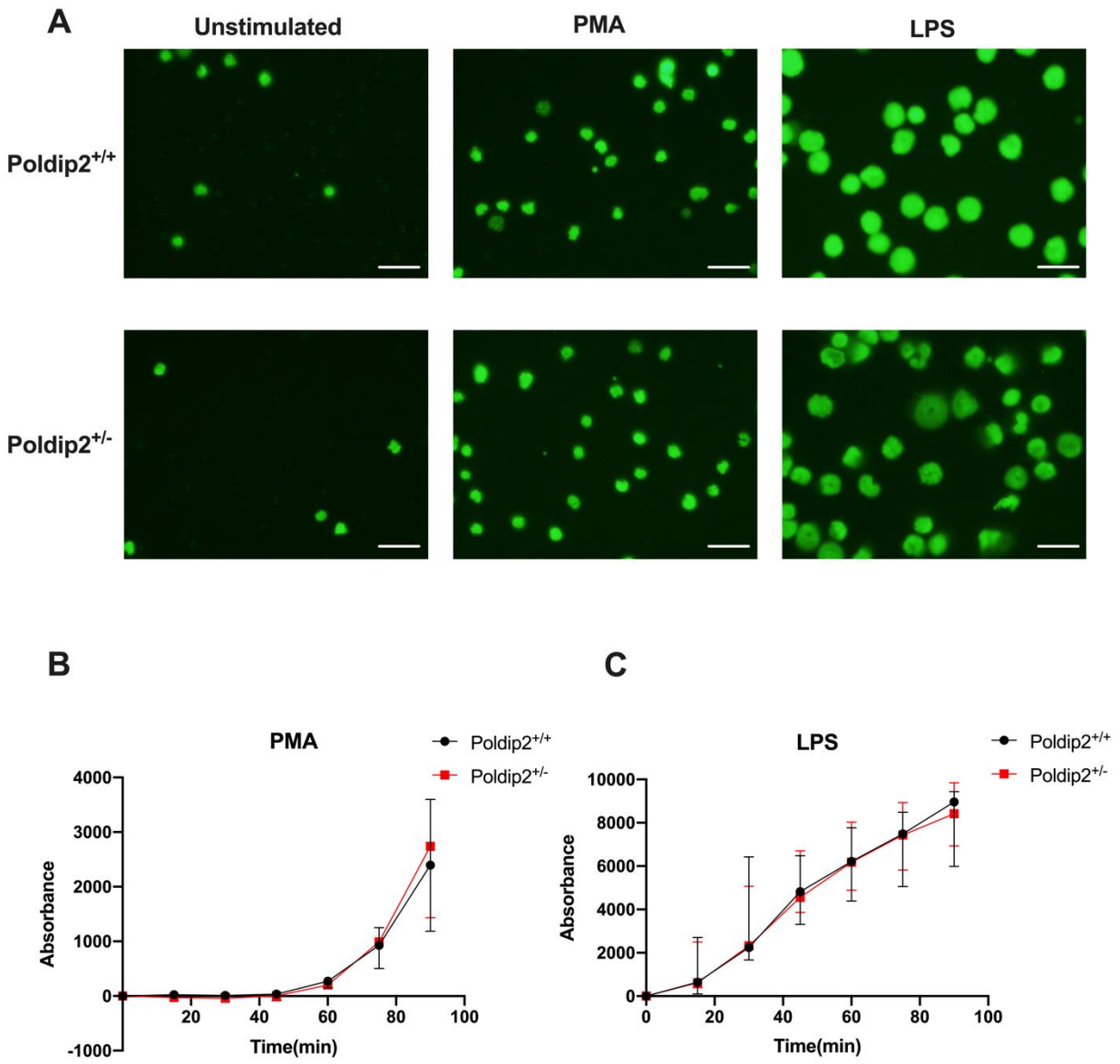
A-B. Intraperitoneal administration of LPS (18mg/kg) induced a significant increase at 18 hours in mRNA expression of chemokines CXCL-1 (A) and CXCL-2 (B) in lung. Data represent medians with 95% confidence intervals (n=6); ** P<0.01 compared to the PBS group of the same genotype (Mann Whitney tests). There was no significant difference between genotypes within the PBS and LPS groups. Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; PBS, phosphate buffered solution; LPS, lipopolysaccharide; CXCL-1, chemokine (C-X-C motif) ligand 1; and CXCL-2, chemokine (C-X-C motif) ligand 2.

Figure S3. Quantification of pro-inflammatory cytokines released by LPS stimulated neutrophils.



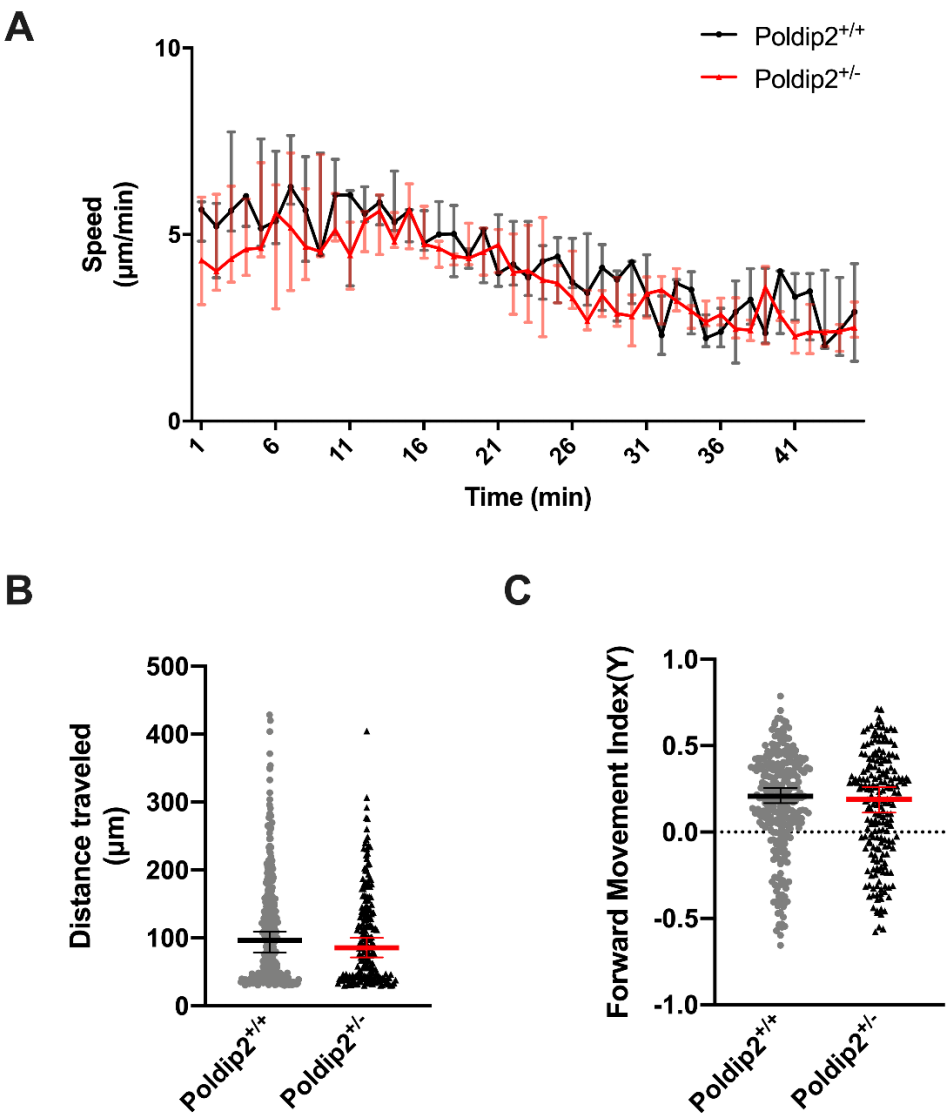
Isolated bone marrow neutrophils from myePoldip2$^{+/+}$ and myePoldip2$^{-/-}$ mice were cultured in RPMI 1640 and stimulated with LPS (1µg/ml) or media alone for 8 hours. Cell supernatant was then collected for TNF-α (A), IL-1β (B) and IL-6 (C) production. Data represent medians with 95% confidence intervals (n=5), ** P<0.01 compared to the PBS group of the same genotype (Mann Whitney tests). Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor α; IL-1β interleukin-1β; and IL-6, interleukin-6.

Figure S4. NET production in response to PMA and LPS stimulations.



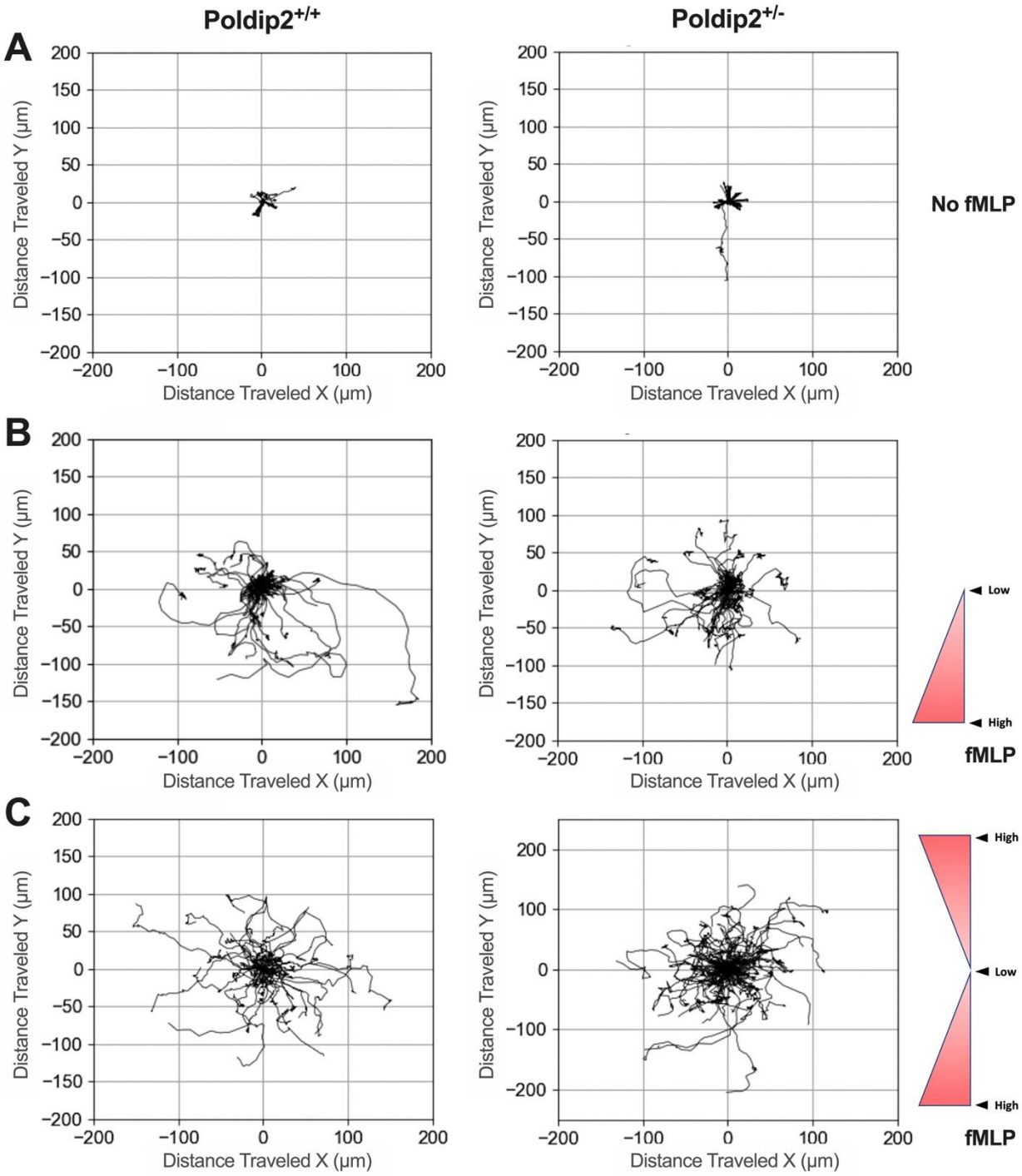
A. Representative pictures of NET formation at 90 min under different conditions. **B-C.** Neutrophils isolated from Poldip2$^{+/+}$ and Poldip2$^{+/-}$ mice were stimulated with 324 nM PMA (B) or 50µg/ml LPS (C), and fluorescence intensity was recorded for 90 min. Scale bars represent 50µm. Error bars represent medians with 95% confidence intervals (n=3). There was no significant difference between genotypes. NET indicates neutrophil extracellular trap (NET); Poldip2, polymerase (DNA-directed) delta interacting protein 2; PMA, phorbol 12-myristate 13-acetate; and LPS, lipopolysaccharide.

Figure S5. Poldip2 knockdown does not affect neutrophil motility.



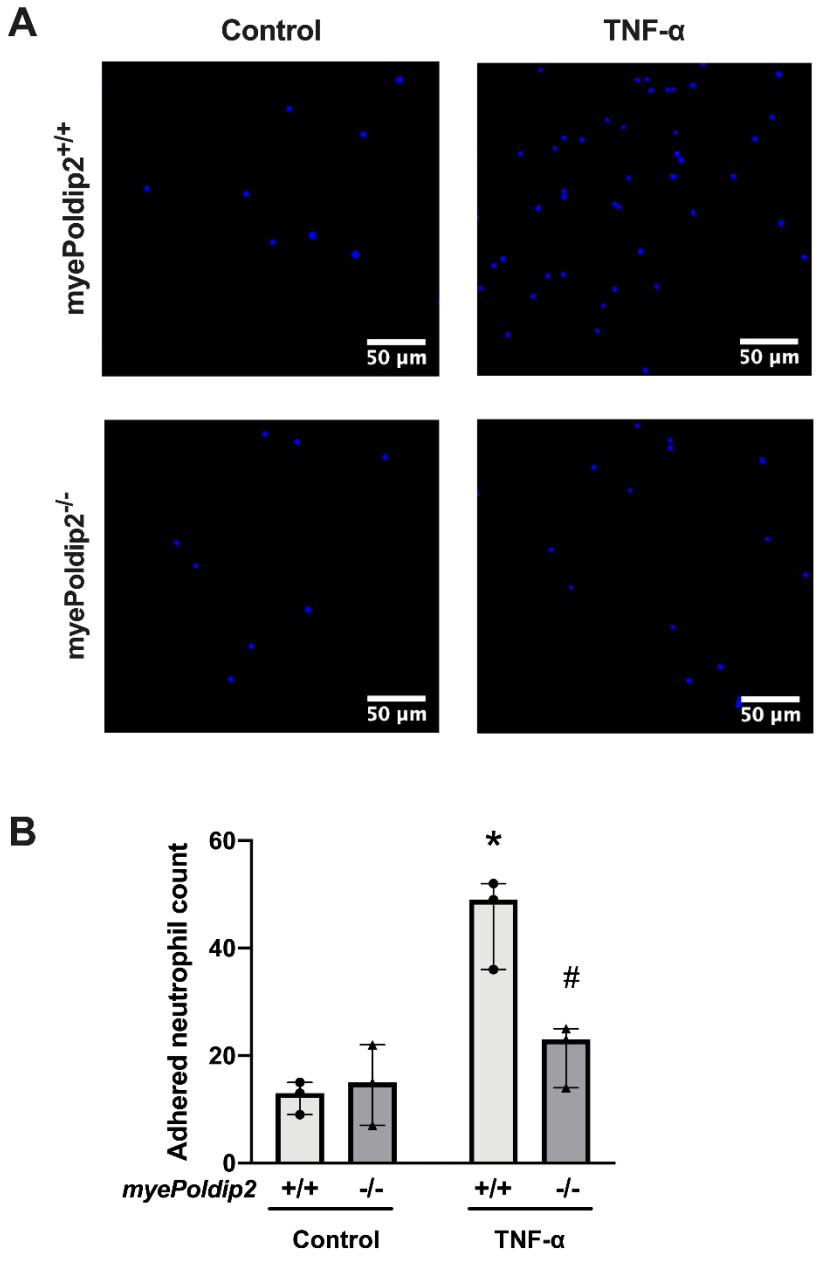
A-C. Isolated bone marrow neutrophils were seeded into µ-slides with 50 µM fMLP on one side. Photomicrographs of neutrophils isolated from Poldip2$^{+/+}$ and Poldip2$^{+/-}$ mice were taken every minute for a period of 45 minutes, starting immediately after plating. The paths of more than 60 neutrophils were recorded in each experiment and 3 independent experiments were performed. (A) Migration speed as a function of time; (B) Total distance traveled; (C) Forward movement index [FMI(Y)] toward fMLP, calculated as Y axis fraction of total distance traveled for 45 min. Single dots represent distance traveled by single cells, lines and error bars represent medians with 95% confidence intervals (n=3), no significant difference was observed between genotypes. Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; and fMLP, N-formylmethionyl-leucyl-phenylalanine.

Figure S6. Trajectories of fMLP-stimulated neutrophils.



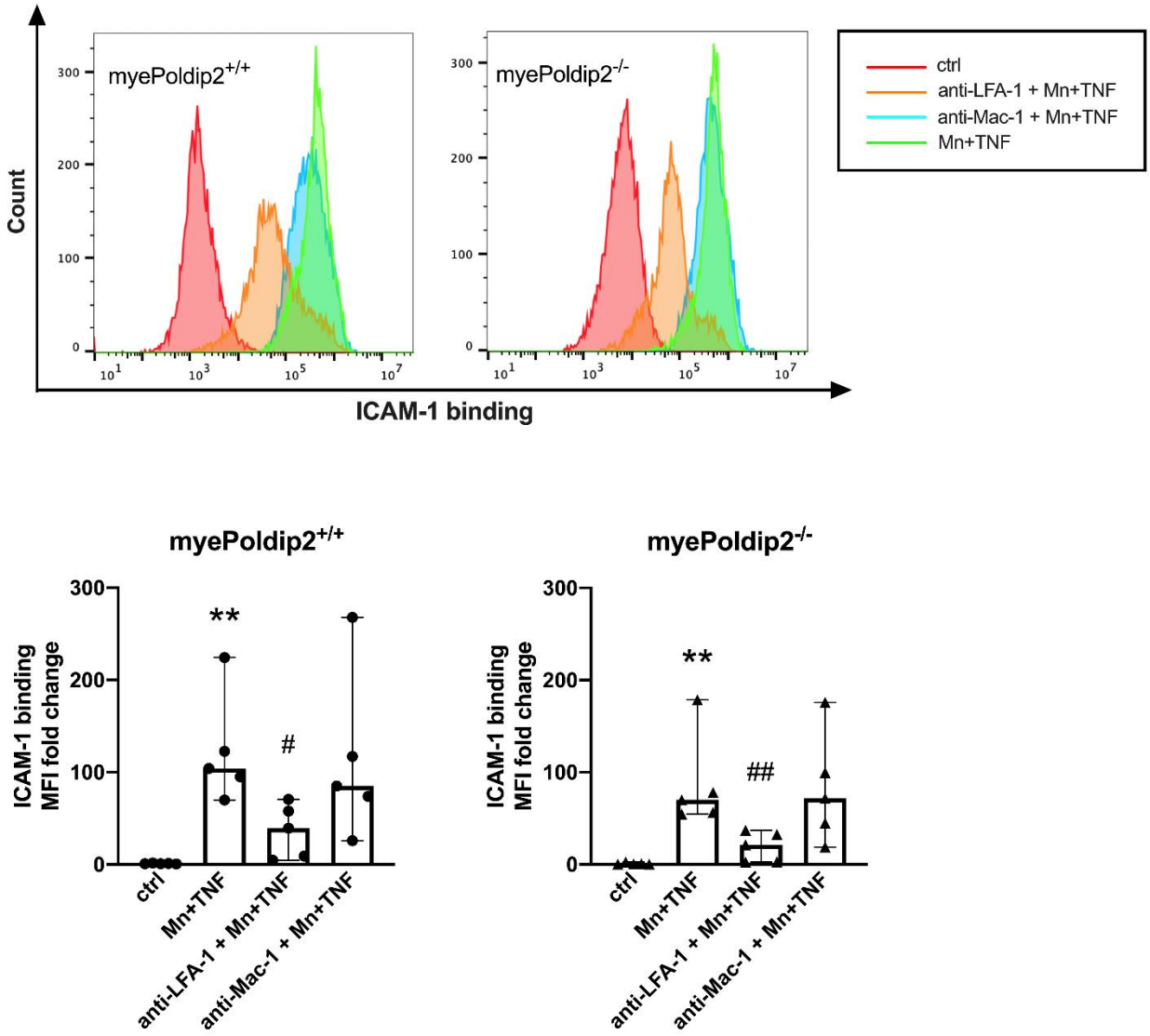
A-C. Representative cell trajectories of bone marrow neutrophils isolated from Poldip2$^{+/+}$ and Poldip2$^{+/-}$ mice, recorded for 45 minutes upon seeding. There was no significant difference in motility between genotypes in response to media alone (A), fMLP (50µM) on one side (B) and fMLP (50µM) on both sides of the assay chamber (C). Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; and fMLP, N-formylmethionyl-leucyl-phenylalanine.

Figure S7. Poldip2 knockdown impairs neutrophil firm adhesion.



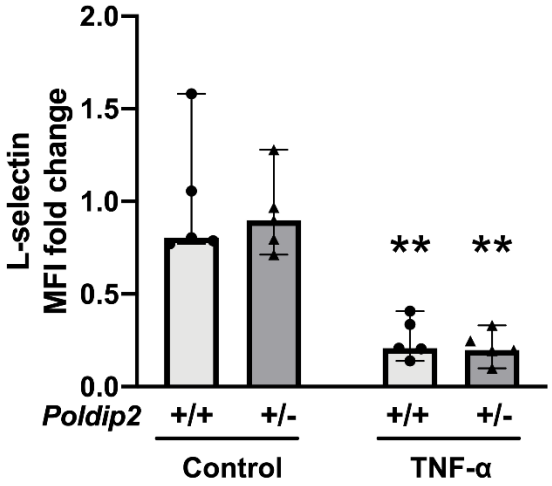
A. Representative pictures of neutrophil adhesion to RPMEC monolayer. Firm adhesion to TNF-α stimulated endothelial monolayers was significantly impaired in myePoldip2$^{-/-}$ neutrophils. **B.** Quantification of results from panel A. Data represent adhered neutrophils relative to control as medians with 95% confidence intervals (n=3); * P<0.05 compared to myePoldip2$^{+/+}$ control group, # P< 0.05 compared to myePoldip2$^{+/+}$ TNF-α group (Mann Whitney tests, one tailed). Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; RPMEC, rat pulmonary microvascular endothelial cell; and TNF-α, tumor necrosis factor α.

Figure S8. Soluble ICAM-1 binding with LFA-1 or Mac-1 blocking antibody.



Neutrophils isolated from myePoldip2+/+ and myePoldip2-/- mice were incubated with either LFA-1 blocking antibody, Mac-1 antibody or HBSS (without Ca^{2+} and Mg^{2+}) prior to Mn^{2+} and TNF stimulation. ICAM-1 binding was quantified by mean fluorescence intensity (MFI). In both genotypes, an apparent decrease of ICAM-1 binding can be identified after blocking LFA-1, which was not observed with Mac-1 blockade. Bar graphs represent medians with 95% confidence intervals (n=5), ** P<0.01 compared to control, # P<0.05, ## P<0.01 compared to Mn+TNF (Mann Whitney tests). Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; Mn, manganese chloride; LFA-1, integrin alpha L; Mac-1, integrin alpha M; HBSS, Hanks balanced salt solution; TNF, tumor necrosis factor α; and ICAM-1, intercellular adhesion molecule 1.

Figure S9. L-selectin shedding in response to TNF-α stimulation.



Isolated bone marrow neutrophils were incubated with 20ng/ml TNF-α or media alone for 1 hour. Cells were then labeled with FITC-L-selectin antibody. Data represent fluorescence intensity (MFI) fold change as medians with 95% confidence intervals (n=5); ** P<0.01 compared to the control group of the same genotype (Mann Whitney tests). No significant difference was noted between genotypes. Poldip2 indicates polymerase (DNA-directed) delta interacting protein 2; and TNF-α, tumor necrosis factor α.